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Green Synthesis of Silver-Decorated Graphene Oxide Nanocomposites Using *Ziziphus spina-christi* Leaf Extract: Characterization and Evaluation of Their Antibacterial Activity

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ABSTRACT

Graphene oxide (GO) was successfully synthesized from graphite using a modified chemical oxidation method and subsequently functionalized with silver nanoparticles through a green synthesis approach employing *Ziziphus spina-christi* (Sidr) leaf extract as a natural reducing and stabilizing agent. The synthesized materials were characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM) to investigate their structural and morphological properties. The characterization results confirmed the successful oxidation of graphite, the presence of oxygen-containing functional groups, increased interlayer spacing, and the formation of exfoliated sheet-like structures with a high surface area. The fabricated Ag-GO nanocomposite exhibited enhanced antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, producing inhibition zones of 11 and 8 mm, respectively. The improved antimicrobial performance was attributed to the synergistic interaction between the graphene oxide matrix and the anchored silver nanoparticles. These findings demonstrate that the greenly synthesized Ag-GO nanocomposite is a promising eco-friendly material for biomedical and environmental applications, particularly in antimicrobial treatments and water purification technologies, while highlighting the potential of green synthesis as a sustainable strategy for nanomaterial production.

1. Introduction

In recent years, the convergence of nanotechnology and biomedical sciences has opened up revolutionary pathways for combating global health challenges. Among various nanomaterials, graphene oxide (GO) - a 2D carbon derivative rich in oxygen-bearing groups has garnered tremendous attention due to its exceptional surface area, robust mechanical properties, and excellent biocompatibility (Zare et al., 2021, Compton & Nguyen, 2010; Dreyer, et al. 2010). Because of these unique structural attributes, GO serves as an ideal substrate for anchoring metallic nanoparticles, preventing their aggregation and enhancing their functional surface area. It is well-established that silver nanoparticles (AgNPs) act as highly effective agents against a wide range of microbial pathogens (Prabhu & Poulose, 2012; Dakal et al., 2016). Combining GO with silver nanoparticles creates a synergistic hybrid nanocomposite that possesses enhanced physicochemical and biological properties, making it a prime candidate for next-generation biomedical applications, particularly in wound healing and sterile coatings. Despite the promising potential of silver-graphene oxide nanocomposites, conventional synthesis methods present significant drawbacks that

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hinder their widespread biomedical application. Standard chemical and physical reduction techniques often rely on harmful reductants, like sodium borohydride, hydrazine, and hazardous organic solvents (Dharaben et al., 2021). These methods not only pose severe environmental risks but also leave chemical residues on the nanomaterial surface, inducing secondary toxicity and limiting their safe application in biological systems. Furthermore, the alarming rise of multidrug-resistant bacterial strains demands the urgent development of highly efficient, biocompatible, and eco-friendly antibacterial agents capable of eradicating pathogens without inducing resistance or toxicity to human cells (Murray et al., 2022). Therefore, developing completely green and non-toxic synthesis routes is imperative for the advancement of safe nanomaterials. Vast scientific efforts have targeted the synthesis and antibacterial mechanisms of silver-decorated graphene oxide. Literature demonstrates that the antimicrobial efficacy of these nanocomposites relies on a dual-action mechanism where GO sheets physically wrap around bacteria to induce membrane stress, while the controlled release of silver ions (Ag^{+}) disrupts cellular DNA and generates destructive reactive oxygen species (ROS) (de Faria, 2014). Recently, the paradigm has shifted toward Green Synthesis to replace hazardous chemicals with natural plant extract (Xin et al. 2022). Various flora, such as green tea, aloe vera, and neem, have been successfully utilized as eco-friendly reducing and stabilizing agents (Iravani, 2011; Park (2014). Utilizing regional plant extracts rich in polyphenols as reducing agents significantly improves the dispersion of silver nanoparticles on the GO matrix, resulting in a remarkable minimum inhibitory concentration (MIC) against pathogenic strains while maintaining low cytotoxicity toward human dermal fibroblasts. Liu et al. (2011); Akhavan & Ghaderi, (2010) demonstrated the biogenic synthesis of Ag/GO nanocomposites using desert plant extracts and reported that the structural synergy between the sharp edges of GO and the released biogenic silver ions leads to complete physical disruption of bacterial cell walls within hours of exposure.

Most previous investigations focused either on the biosynthesis of AgNPs alone or on Ag–GO composites synthesized using different plant extracts without evaluating the role of *Ziziphus spina-christi* in producing a highly dispersed Ag-decorated GO nanostructure for photocatalytic applications. To address these challenges, this study proposes a completely green, an environmentally benign strategy to prepare silver-anchored graphene oxide (GO) nanocomposites. GO is initially derived from graphite utilization a controlled chemical oxidation method, followed by the advancement of a hybrid nanocomposite through the incorporation of silver nanoparticles via a biogenic approach employing Sidr (*Ziziphus spina-christi)* plant-derived solutions acting as ecologically benign reductants. To thoroughly investigate the morphology, chemical structure, and functional attributes of the prepared hybrid material, we utilized a combination of SEM, XRD, and FT-IR spectroscopies. Finally, the Antibacterial potency of the prepared nanocomposite is evaluated, demonstrating significant efficacy, especially toward *Staphylococcus aureus* and *Escherichia coli*, due to the interfacial synergy established between AgNPs and the GO. The core innovation and new value of this research lie in the novel utilization of Sidr plant extract as reducing agent to engineer Ag-GO hybrid structure. Sidr extract is exceptionally contains bioactive molecules, like flavonoids, polyphenols and saponins, which efficiently reduce silver ions while forming a natural stabilizing layer that prevents nanoparticle agglomeration. This study provides valuable insights into the field by delivering a cost-effective, scalable, and non-toxic protocol that maximizes the synergistic antibacterial effect between GO and AgNPs, offering a potent and environmentally safe alternative to conventional chemical disinfectants and antibiotics.

2. Methodology

3.1. Materials and Reagents

Graphite powder, $NaNO_3$, H_2SO_4, $KMnO_4$, H_2O_2 (30%), ethanol (C_2H_5OH), silver nitrate ($AgNO_3$), and sodium hydroxide (NaOH) were

purchased from standard commercial suppliers and used without further purification. Throughout the experimental trials and washing stages, deionized water was employed exclusively. Dried leaves of the Sidr (*Ziziphus jujuba*) plant were sourced locally to be utilized as the biogenic reducing and stabilizing agent. The University of Hafr Al-Batin's Department of Biology (Saudi Arabia) supplied the *E. coli* and *S. aureus* strains used in this study, which were cultured on prepared nutrient agar plates for antibacterial testing

3.2 Synthesis of Graphene Oxide

Based on a Hummers method, a chemical oxidation strategy was employed to synthesize GO from pristine graphite powder. In a representative procedure, a mixture containing $NaNO_3$ (2.5 g) and powdered graphite (5g) were introduced into a glass beaker. The resulting solution was transferred into an ice bath for 30 min to maintain a low temperature during the initial stages of reaction. Subsequently, 115 mL of concentrated H_2SO_4 was introduced drop by drop during constant and intense agitation, and the mixture was allowed to react for another 30 min. Then, 15 g of $KMnO_4$ was slowly introduced in small portions while strictly controlling the temperature to prevent exothermic overheating and potential hazards. Following the addition, the resulting blend was moved into a water bath under ongoing stirring continuously (30 min) at 35° C. Afterward, 500 mL of DIW was cautiously added dropwise, and the temperature of the system was raised to 95 °C and maintained at this temperature for 15 min to complete the oxidation process. To terminate the oxidation process, 10 mL of 30% H_2O_2 was added into the suspension, resulting in an instantaneous color change that confirmed the successful conversion to highly oxidized graphene oxide. The reaction medium was left to attain room temperature spontaneously, filtered, and then purified by rinsing through multiple washing cycles involving ethanol and DIW. The collected substance was then placed in a laboratory oven at 60 °C until a constant weight was achieved, and then ground into a fine brown GO powder (Zaaba et al, 2017).

3.3. Preparation of Precursor Solutions

To prepare the silver nitrate precursor solution, 150 mg of $AgNO_3$ powder was precisely weighed before being dissolved within a volumetric flask (100 mL) using DIW. The flask was stirred continuously until complete dissolution occurred and was subsequently wrapped in aluminum foil to protect the light-sensitive silver ions from photo-reduction. Separately, finely prepared GO powder (60 mg) was integrated into 100 mL of DIW to fabricate the aqueous GO suspension. The dispersion was subjected to microwave heating and then magnetically stirred for 30 min to ensure uniform exfoliation and homogeneity. Finally, the $AgNO_3$ solution was added dropwise into exfoliated GO suspension under constant magnetic stirring, and the mixture was kept under agitation for 30 min to facilitate the electrostatic adsorption of silver ions onto the Oxygenated moieties attached to the GO sheets (Figure1).

3.4 Phytochemical Extraction of Sidr (Ziziphus spina-christi) Leaves

To prepare the Sidr leaf extract, a 2 g sample of pre-dried, finely pulverized Sidr leaves powder was introduced into a glass flask filled with 120 mL of ethanol (solid-to-solvent ratio of 1:60, w/v**)**. The mixture was processed under continuous stirring using a hot-plate magnetic stirrer maintained at 60 for 58 min to maximize the extraction yield of essential bioactive substances like flavonoids, polyphenols, and saponins, following the extraction protocol reported by Mahdavi et al. (2013). Once the extraction process was complete, the resulting solution was cooled down to room temperature and subsequently passed through fine filter paper to eliminate insoluble plant residues. The collected ethanolic filtrate was evaporated and dried in a drying oven to yield a solid extract. To prepare the working stock solution, 50 mg of the dry biogenic extract was re-dissolved in 100 mL of deionized water (Figure 1).

3.5 Green Synthesis of Silver-Decorated Graphene Oxide (Ag-GO) Nanocomposite

The biogenic reduction and decoration process was carried out by loading the prepared Sidr plant extract into a burette and introducing it dropwise into the mixed Ag^{+}/GO precursor suspension at a slow and controlled rate under rapid magnetic stirring. To optimize the reduction rate and stabilize the newly formed hybrid nanomaterial, alkalinization of the reaction medium to pH 8 was achieved by dropwise addition of 0.1 M NaOH under constant pH-meter monitoring. The solution was left under continuous magnetic stirring for 60 min at 25 ± 2 °Cto ensure complete reduction of silver ions into silver nanoparticles (AgNPs) immobilized on the GO surfaces (Al-Askar & Ghoneem, 2021). To harvest the final Ag-GO nanocomposite, the mixture was centrifuged, and the precipitate was subjected to several washing cycles with deionized water and ethanol for purification. The isolated hybrid powder was then obtained after drying the sample inside an oven.

3.6 Characterization Framework

The structural, functional, and morphological properties of the fabricated materials were systematically analyzed utilizing advanced analytical equipment:

FT-IR: Employed to confirm the existence of specific chemical bonds in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans.

XRD: Carried out to assess the crystalline phase, purity, and changes in the interlayer spacing (d spacing) of the carbon sheets and was performed using Cu Kα radiation (λ = 1.5406 Å). The diffraction patterns were recorded over a 2θ range of 5–80° with a step size of 0.02° under standard operating conditions.

SEM: Executed to characterize the surface features and microstructural properties, sheet like exfoliation structure, high surface area, and the spatial distribution of the decorated silver nanoparticles over the GO template.

3.7 Evaluation of Antibacterial Activity

Following the protocol described by Olaru et al. (2024), the antibacterial performance of the green-synthesized Ag-GO hybrid nanocomposite was tested via agar well diffusion assay technique. The assay targeted *S. aureus* (Gram-positive) and *E. coli* (Gram-negative), utilizing bacterial suspensions calibrated to a uniform density. Initially, bacterial cultures were prepared and calibrated to match a standard microbial concentration. The bacterial inoculum was adjusted to 0.5 McFarland (≈1.5×10^{8} CFU/mL). To maintain sterility, the autoclaved nutrient agar medium was cast into individual Petri plates and allowed to cool until completely solid within a laminar flow hood. The culture was uniformly spread over the surface of the agar matrix. Subsequently, small circular wells were carefully bored into the agar layers using a sterile cork borer. To prepare the nanocomposite stock solutions, a concentration of 1 mg/mL was achieved using deionized water, followed by a 30-minute sonication process and then loaded into the corresponding wells. The agar plates were then subjected to thermal incubation at a constant 37 °C for a 24-hour period. Post-incubation, the clear zones of inhibition around each well were carefully evaluated and recorded in millimeters (mm) to quantify the synergistic antibacterial efficacy of the hybrid material.

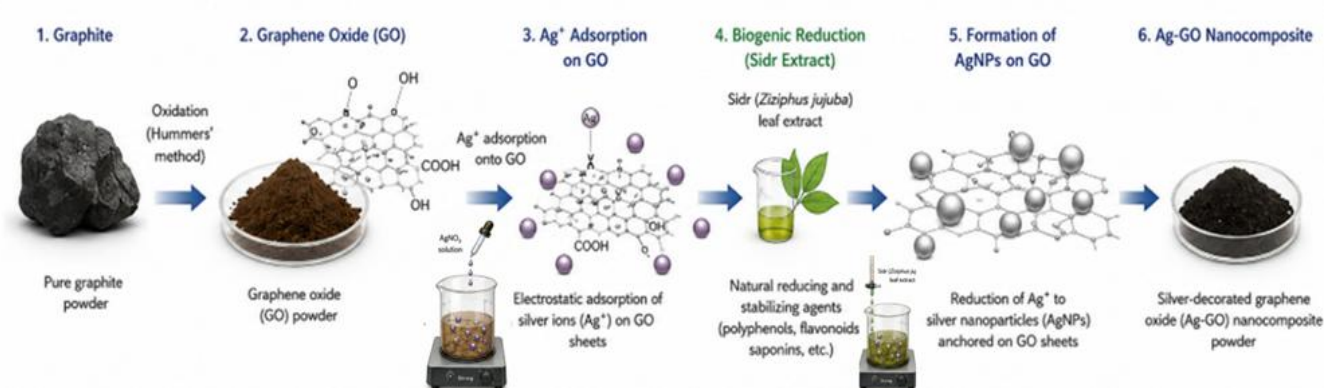

Figure 1. A diagrammatic representation of Ag-GO nanocomposite through biogenic reduction of Ag^+ using Sidr (*Ziziphus spina-christi)* leaves extract.

3. Results and discussion

3.1 XRD Analysis

XRD pattern of of the synthesized GO is depicted in Figure (2). A strong diffraction peak is observed at approximately 2θ =10°, corresponding to the (001) crystallographic plane of graphene oxide. This characteristic peak confirms the successful oxidation of graphite and the formation of GO. According to Bragg's law, the calculated interlayer spacing (d-spacing) was approximately 0.88 nm, which is significantly larger than that of pristine graphite (~0.34 nm). The shift from the characteristic graphite peak at around 26° to 10° indicates a significant increase in the interlayer spacing, resulting from the presence of oxygenous groups and water molecules intercalating the graphene layers. The broadening of the diffraction peak suggests a decrease in crystallinity and the formation of exfoliated graphene oxide sheets (Stankovich et al., 2007). These results confirm the successful synthesis of graphene oxide.

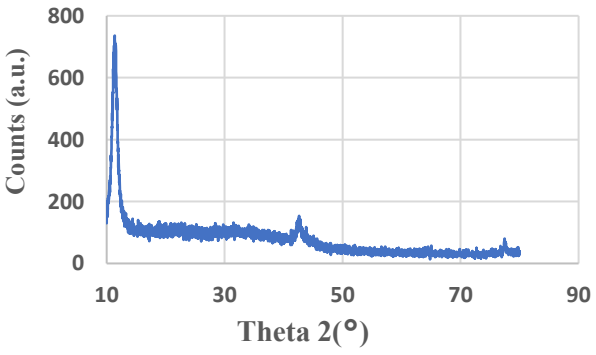

Figure 2. XRD spectrum of GO

3.2 FTIR Analysis

Figure (3) shows the FT-IR spectrum of the synthesized graphene oxide sample. Several characteristic absorption bands were observed, confirming the successful oxidation of graphite and the formation of graphene oxide. Within the 3200–3700 cm^{-1} range, the prominent, wide absorption band signifies the stretching mode of O–H (hydroxyl) groups. The stretching modes of carbonyl (C=O) functional groups are reflected by the peak located within the 1700–1800 cm^{-1} range, originating from carboxylic functionalities. An absorption band at approximately 1600 cm^{-1} signifies the C=C vibration of pristine graphitic patches. Concurrently, the signals detected between 1000 and 1300 cm^{-1} correspond to C–O bonds in alkoxy and epoxy moieties (Guo et al., 2012). The transformation of graphite to GO via successful oxidation is validated by the appearance of these oxygen-bearing moieties.

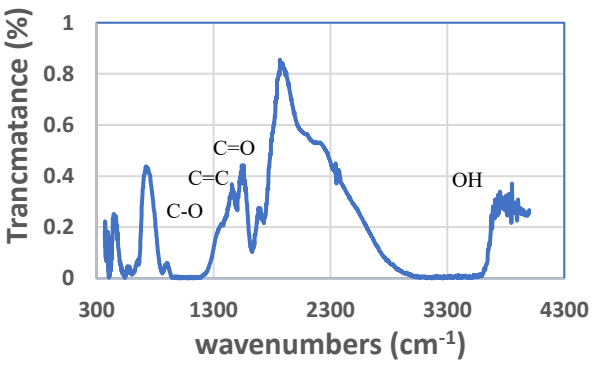

Figure 3. FTIR spectrum of GO

3.3 SEM Analysis

The SEM micrographs reveal a clear morphological difference between pristine graphene oxide Figure (4a) and the silver-decorated graphene oxide (Ag-GO) nanocomposite Figure (4b). The synthesized GO exhibits a characteristic layered and wrinkled sheet-like morphology, appearing as thin, irregularly stacked flakes with rough surfaces and folded edges. This crumpled structure is attributed to the oxidation and exfoliation of graphite layers during the synthesis process. The presence of overlapping and loosely packed sheets indicates an increased surface area and confirms the successful formation of graphene oxide nanosheets. These morphological features are consistent with previously reported graphene oxide structures (Marcano et al., 2010). In contrast, the Ag-GO nanocomposite displays a rougher and more compact morphology, with numerous granular particles distributed over the GO sheets. These particles are due to successful deposition of Ag nanoparticles on the GO surface, resulting in increased surface roughness and partial sheet aggregation. The observed morphological changes provide strong evidence for the successful anchoring of silver nanoparticles onto graphene oxide through the green synthesis process using *Ziziphus spina-christi* (Sidr) leaf extract.

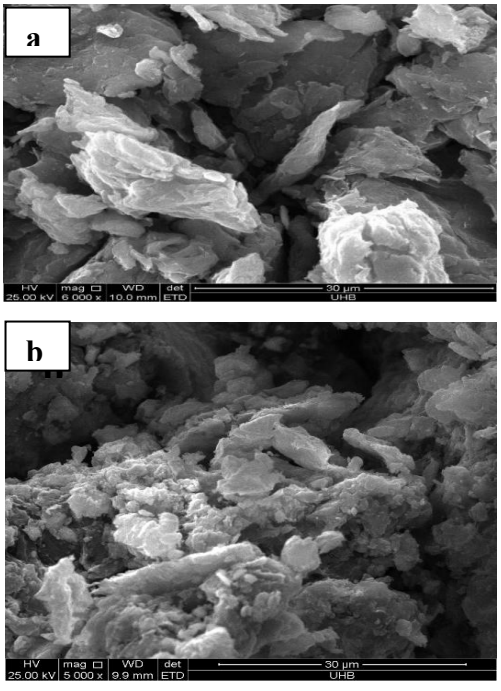

Figure 4. SEM images of a: GO and b: Ag-GO

3.4 Antibacterial Activity and Synergistic Mechanism

The biocidal efficacy of the synthesized materials was qualitatively evaluated against model bacterial pathogens, namely *E. coli* alongside *S. aureus*, as illustrated in Figure 5. The nutrient agar plates were embedded with three distinct wells labeled 1, 2, and 3, corresponding to the graphene oxide (GO), deionized water, and the Sidr extract silver-decorated graphene oxide nanocomposite (GNP-AgNPs) respectively.

The findings indicate that the highest antibacterial efficacy was exhibited by the GNP-AgNPs nanocomposites (sample 3) against *S. aureus*, recording an inhibition zone diameter of 11 mm (Figure 5b). In contrast, a lower susceptibility was noted for E. coli, which displayed a clearance zone of approximately 8 mm. Pure GO (label 1) showed zones of inhibition against *S. aureus* and *E. coli* with a zone diameter of 7 mm and 5 mm, respectively (Figure 5). This intrinsic antibacterial trait of pristine GO is primarily assigned to a mechanical nanoknife mechanism, wherein the sharp, atomically thin edges of the exfoliated graphene sheets induce physical membrane stress upon direct cellular contact (Akhavan & Ghaderi, 2010).

When comparing phenotypic susceptibilities, the fabricated hybrid nanocomposite demonstrated markedly higher antibacterial response against the Gram-positive strain than against the Gram-negative strain. This prominent variation is deeply rooted in the fundamental structural discrepancies of the bacterial cell wall architectures: *E. coli* membrane barrier. The cellular envelope of *E. coli* is characterized by a highly complex, multi-layered structure consisting of a thin peptidoglycan layer shielded by an asymmetric outer lipopolysaccharide (LPS) membrane. This outer lipid bilayer acts as an effective permeability barrier, restricting the continuous penetration of released silver ions and bulkier nanocomposite sheets into the cytoplasm (Dakal

et al., 2016). Gram-Positive Membrane Susceptibility (*S. aureus*): Conversely, Gram-positive bacteria lack this protective outer lipophilic membrane, possessing instead a porous, thick peptidoglycan network. This macromolecular mesh allows the rapid diffusion of biogenic nanoparticles and silver ions, making them more vulnerable to intracellular disruption (Tang & Lv, 2014).

The outstanding antibacterial activity observed in Well 3 is fundamentally governed by a profound synergistic effect between the GO matrix and the anchored silver nanoparticles. Within this hybrid framework, the high surface area of the GO sheets serves as an excellent dispersant template that effectively minimizes the self-aggregation of the anchored AgNPs, thereby maintaining a high surface-to-volume ratio. Mechanistically, as the GO carrier physically binds to and compromises the bacterial cell envelope, it facilitates a targeted, localized release of Ag^+ ions. These silver ions are believed to interact with intracellular thiol (-SH) and phosphorus groups, leading to DNA condensation, protein denaturation, and the overproduction of cytotoxic Reactive Oxygen Species (ROS) (Prabhu & Poulose, 2012). This dual-action pathway is believed to enhance the antibacterial activity of the nanocomposite. Consequently, these robust outcomes highlight the potential of the fabricated green Ag-GO nanocomposite to serve as a highly efficient, sustainable, and eco-friendly biomaterial for advanced biomedical and environmental decontamination applications.

Figure 5. Zone of inhibition generated by GO and GNP-AgNPs in (a) *E. coli*, (b) *S. aureus*

As shown in Table X, the antibacterial activity of the Sidr-mediated Ag-GO nanocomposite is comparable to reported GO–Ag systems, owing to the synergistic effects of GO nanosheets and Ag nanoparticles through membrane disruption, Ag^+ release, and ROS generation.

Table (1): Comparison of antibacterial activity of Ag-GO nanocomposites with recently reported studies

Nanocomposite	Tested bacteria	Antibacterial effect	Reference
GO–Ag nanocomposite	*Escherichia coli* and *Staphylococcus aureus*	Enhanced bactericidal activity compared with GO and Ag nanoparticles; reported inhibition efficiencies of 73% (*E. coli*) and 98.5% (*S. aureus*)	Vi et al., 2020
GO–Ag nanocomposite	Multidrug-resistant *E. coli*	Strong antibacterial activity; bacterial viability significantly reduced at low nanocomposite concentration	Chen et al., 2020
GO/Ag nanocomposite	*S. aureus*, *Bacillus subtilis*, *E. coli*, and *Salmonella enterica*	Potent antibacterial performance confirmed by MIC and time-dependent antibacterial assays	Sharmila et al. 2024)
GO-supported Ag nanomaterial	*E. coli* and *S. aureus*	High antibacterial activity due to stable Ag nanoparticle dispersion on GO sheets	Liu et al, 2023
Sidr-mediated Ag-GO nanocomposite	*E. coli* and *S. aureus*	Inhibition zones of 8 mm and 11 mm, respectively	This work

4. Conclusions

The study comprehensively achieved the oxidative synthesis of GO from raw graphite, establishing a highly reactive precursor that was subsequently utilized as a support matrix for the green synthesis of silver-decorated graphene oxide (Ag-GO) nanocomposites using *Ziziphus spina-christi* (Sidr) leaf extract. SEM analyses confirmed the successful formation of GO and the effective incorporation of silver nanoparticles onto its surface. The synthesized Ag-GO nanocomposite demonstrated improved antibacterial activity against Gram-negative bacteria alongside Gram-positive strains, with higher inhibition zones than pristine GO. The observed enhancement confirms the beneficial

role of silver nanoparticle incorporation in improving the antimicrobial performance of graphene oxide. These findings demonstrate that the developed Ag-GO nanocomposite is an environmentally friendly and effective antimicrobial material with promising potential for future antimicrobial and environmental applications.

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